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FOREWORD

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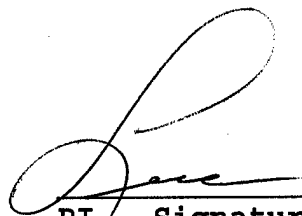
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Fellowship Progress Report

Specific Aims-The Regulation and Function of the Nuclear Receptor Corepressor SMRT in Human Breast Cancer Cells

1. I will investigate the expression of SMRT in various human breast cancer cell lines and determine if retinoic acid, estrogen, and/or 4HT treatment are involved in the regulation of SMRT RNA/protein levels. This data will be compared to the levels of RAR and ER in each cell line.
2. I will investigate the interactions between SMRT and ER upon treatment with hormone or anti-hormone in yeast-two-hybrid, mammalian-two-hybrid, and co-immunoprecipitation experiments in order to determine if SMRT is a link between RAR and ER-driven gene regulation.
3. I will investigate the mechanism of 4HT- and RA-mediated inhibition of breast cancer cell growth in cell proliferation assays. The ability of an overexpressed dominant negative SMRT mutant(C.SMRT) lacking the repression domain to block growth inhibition by 4HT and atRA will be determined.

In the course of preparing and submitting my proposal to investigate the role of the nuclear receptor corepressor SMRT in human breast cancer as outlined above, several projects analyzing the function of SMRT and the related corepressor NCoR, as well as the nuclear receptor coactivator Rac3(p/CIP/ACTR/AIB1) were being undertaken. Significant progress has been made in the past year towards better understanding the function of these cofactors and the mechanism by which they regulate the activity of nuclear receptors such as the estrogen receptor. This progress will be highly beneficial in elucidating the role that nuclear receptor signaling plays in breast carcinogenesis.

1. Identification of a Novel SMRT/NCoR interacting protein

In the absence of ligand, steroid hormone receptors interact with the highly related corepressors SMRT and NCoR to actively repress transcription below the basal level. These cofactors interact with the unliganded receptor *in vitro* and *in vivo*, enhance transcriptional repression by the receptor, and dissociate away upon hormone binding to the receptor. The precise mechanism by which the SMRT/NCoR-receptor complex represses transcription is not completely understood, but likely involves the recruitment of an additional complex, which includes the corepressor mSin3, the histone deacetylase HDAC, and additional factors. I am currently characterizing a novel SMRT/NCoR interacting protein termed SNIP, which interacts with corepressors *in vitro* and *in vivo*, and also represses transcription. This factor may be an additional member of the corepressor complex or a transcription factor regulated by SMRT and NCoR.

SNIP was isolated in a yeast-two-hybrid library screen using the first repression domain of NCoR(NRD1/NcoR 1-312) as bait. SNIP interacts specifically with NCoR in yeast-two-hybrid, mammalian-two-hybrid, and *in vitro* assays. SNIP also interacts with a recently cloned, longer isoform of SMRT, termed hSMRTE(Park, et al. *PNAS* 96:3519(1999)), which contains an extended N-terminal domain with homology to the N-terminus of NCoR(Fig.1). These interactions were mapped and found to be dependent upon predicted α -helical domains within NCoR, hSMRTE, and SNIP via site-directed mutagenesis. Upon fusing SNIP to the Gal4 DNA binding domain and transfecting it into HeLa cells along with a luciferase reporter driven by Gal4 binding sites, SNIP was found to repress transcription when compared to the Gal-DBD alone(Fig.2). It is proposed that this repression activity is due to the recruitment of the NCoR/SMRT corepressor complex *in vivo*. Ongoing experiments are aimed at investigating the biological function of SNIP in both hormone receptor signaling and the regulation of nuclear receptors by SMRT/NCoR. Since recent data implicate altered NCoR levels as possibly regulating the function of tamoxifen as an agonist versus an antagonist(Lavinsky, et al. *PNAS* 95:2920(1998)), it will be critical to determine the physiological function of SNIP in the hopes of identifying the potential role that the SMRT/NCoR corepressor complex plays in breast cancer development and treatment.

2. Regulation of Nuclear Receptor Function by the Coactivator Rac3(AIB1)

Hormone binding by nuclear receptors triggers the dissociation of the SMRT/NCoR corepressor complex and subsequent recruitment of a coactivator complex. This complex includes members of the SRC1 family of coactivators, such as Rac3, and also the general coactivators CBP/p300. These coactivators enhance transcriptional activation by liganded hormone receptors via mechanisms that include histone acetylation. At the time that Rac3 was cloned in our laboratory(Li, et al. *PNAS* 94:8479(1997)), the same gene was also cloned as AIB1(amplified in breast cancer-1) in a screen for genes with elevated expression in human breast cancers(Anzick, et al. *Science* 277:965(1997)). Thus it is likely that nuclear receptor coactivators also play a role in human breast cancer.

Intriguingly, these coactivators all share 6-7 common motifs containing the core consensus sequence LXXLL, where L is leucine and X is any amino acid. Work in our laboratory and others has demonstrated that specific motifs are critical to coactivator-receptor interactions or coactivator transcriptional activation. Other studies(McInerney, et al. *Genes & Dev.* 12:3357(1998)) on SRC1 also present a case for a receptor specific code of interaction, where different nuclear receptors require different LXXLL motifs in order to bind coactivator. In light of this, I am investigating the motifs involved in both Rac3/AIB1 interactions with receptors and Rac3/AIB1 transcriptional activation in the hopes of better understanding the function of this cofactor in regulating nuclear receptor signaling and its contribution to breast cancer development and pathology.

To accomplish this, I have done a detailed mapping of the region of Rac3/AIB1 that is involved in nuclear receptor binding. An entire panel of Rac3/AIB1 fragments, in total comprising the full-length protein, was purified as GST fusion proteins and probed with *in vitro* translated ³⁵S-labelled nuclear receptors. Figure 3 represents an analysis of the Vitamin D receptor(VDR) and the estrogen receptor beta(ER β). The VDR interacts specifically with Rac3 613-752 and 723-1034 in a ligand-dependent manner. The 613-752 fragment contains LXXLL motifs 1,2,3 while the 723-1034 fragment contains only motif 3. This data implicates motif 3 as being critical to Rac3-VDR interaction. A different picture is observed with ER β , for in addition to the 613-752 and 723-1034 fragments, it also interacts with Rac3 1-407, which does not contain any LXXLL motifs, and 342-646, which contains motif 1. These differences in the Rac3

binding surface for the VDR and ER β may represent differential regulation of nuclear receptors by Rac3/AIB1. Ongoing experiments include site-directed mutagenesis of these LXXLL motifs in order to determine which motifs are involved in coactivation of nuclear receptor function by Rac3/AIB1. Based on the ability of Rac3/AIB1 to interact with and enhance transcription by the estrogen receptor and by its overexpression in breast tumors, this coactivator is likely an integral component of the estrogen signaling pathway. Continued work in our laboratory on the function of Rac3/AIB1 will hopefully lead to a better understanding of steroid hormone receptor involvement in human breast cancer.

Fig. 1

SNIP Interacts with NCoR and hSMRTe

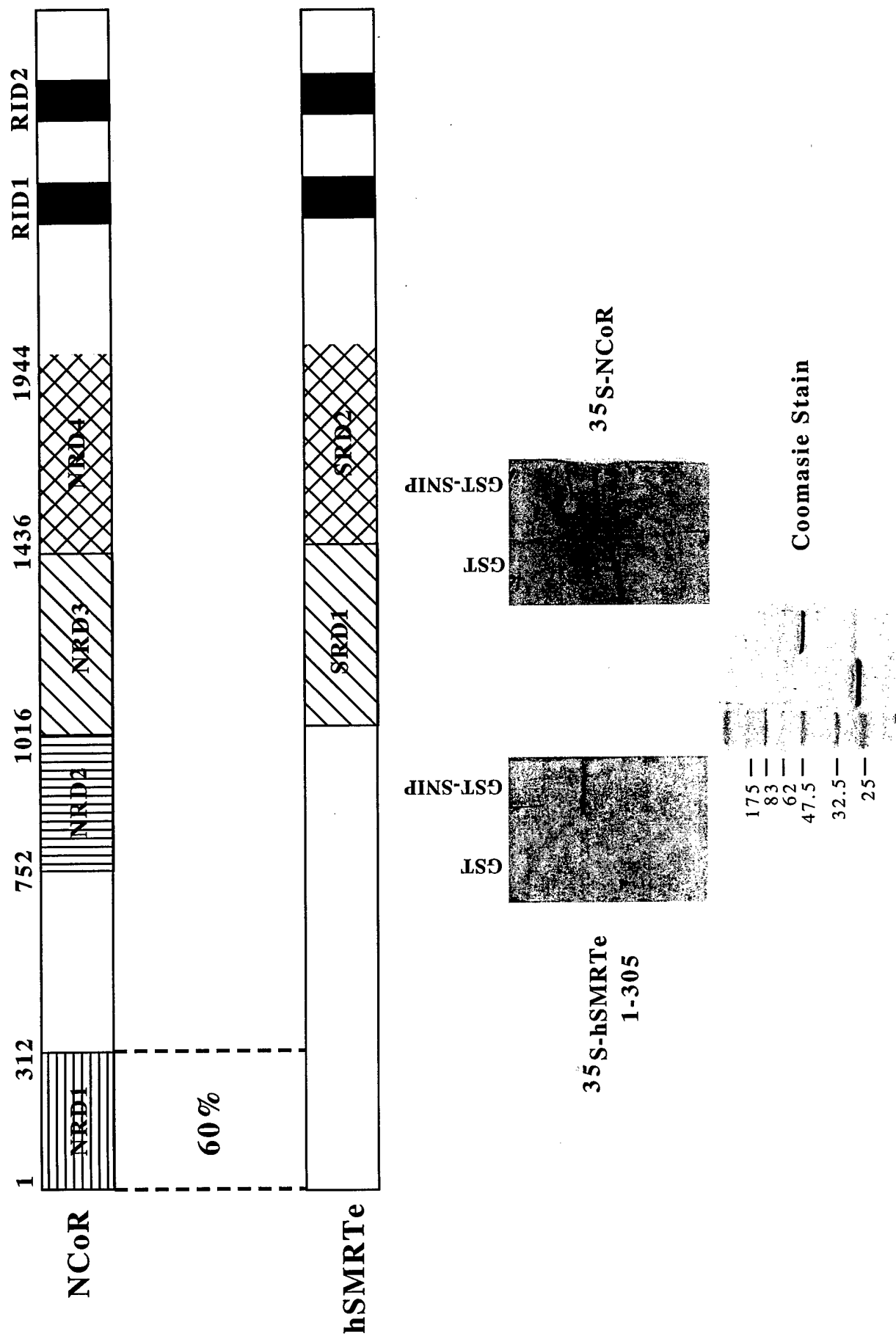
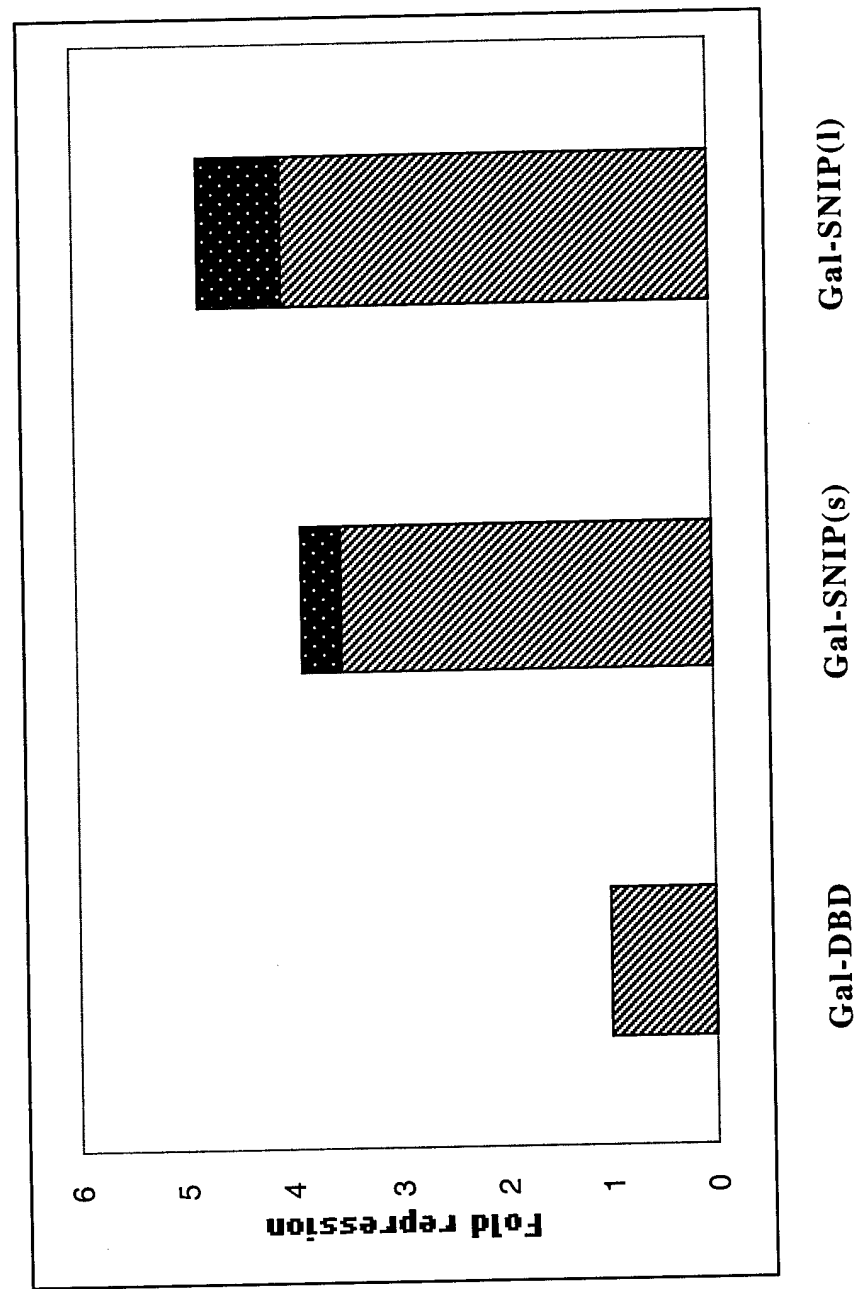


Fig. 2

SNIP represses transcription



Each Gal-DBD fusion was transfected into HeLa cells along with a β -galactosidase construct and a luciferase reporter construct harboring 4 Gal-DBD binding sites. Upon harvesting, luciferase activity was measured and normalized to β -galactosidase activity.

Fig. 3

VDR/ ER β Interactions with Rac3 In Vitro

